當歸的生產及品質提升之研究

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摘 要

當歸是繖花序科多年生草本植物，使用部位爲植物的乾燥根，在神農本草經收載列爲中品。主要的功能爲補血、行血、潤腸、調經，其適應證爲血虛頭痛、心腹諸痛、大便燥結、婦女經閉、經來腹痛、崩漏。自古以來在傳統中醫藥方劑中大量的被使用，是重要的藥材之一。

本研究共分成四個架構對當歸進行探討，其一爲栽培條件與生長、生產及品質之間的關係，從採種、育苗、肥培管理到環境條件等，進行一系列的生理、生態之研究，探討最適當的栽培方式。其二爲抽苔防止法之建立，當歸具有容易抽苔、開花之特性，抽苔後的植物體會引起生長、生理活性的低下，甚至隨著木質化的進行根部會停止肥大，導致植株枯萎死亡造成收穫上的損失，是栽培上的一大障礙。本研究中建立了切根法，不僅可提高抽苔防止效果並可促進根部的肥大，提高產量。其三爲理化學的品質評價，從當歸的種子中單離純化出 ligustilide ，利用螢光檢測器建立高效液相層析儀 (HPLC) 的定量分析方法。並利用薄層層析儀 (TLC) 及 HPLC探討 ?-sitosterol 、 stigmasterol 、 cholesterol 及 campesterol 等 sterol 類的成分與 bergapten 、 umbelliferone 、 scopoletin 、 xanthotoxin 、 isopimpinellin 及 imperatorin 等 coumarin 類的成分在各種不同當歸及不同部位之組成及分佈。最後爲利用組織培養方法達到種苗大量增殖，以未熟種子、葉、葉柄爲材料，利用各種培養基及荷爾蒙的組合，探討誘導直接或間接不定胚 (somatic embryogenesis) 的最適條件，並成功的誘導出植物體，經馴化後可達大量增殖之目的。

Studies on the production and the improvement in quality of Angelica acutiloba Kitagawa were carried out in order to verify the effect of the production technique under study.

The study yielded the following results:

Ⅰ .Studies on plant growth, production and improvement in quality in different cultivation conditions.

1.The investigation focused on the relationship of the cultivation environment to the character of A. acutiloba Kitagawa seedlings1).

The results suggest that we can easily produce mid-size A. acutiloba seedlings (root diameter 8(9mm) when the A. acutioba plant was cultivated at 4 plant per Wagner's pot (1/2000 a), under 60 % shading conditions, and when nitrogenous fertilizer in the form of ammonium nitrate or ammonium sulfate is used.

2.Comparison of the effect on plant growth, yield, and quality of variations in location of terminal flower, grade of maturity, and seed coat color in seeds from several production years.

The results suggest that in the cultivation of "Dang-guei", the optimal effect in terms of plant growth, yield, and crude drug quality can be realized when the seeds are collected as third year main stem terminal flower seeds at the yellow ripe stage, and when the seed coats were green.

3.The effects of different manurial elements on the plant growth, yield. extract contents, and ligustilide and butylidene phthalide contents of A. acutiloba were investigated using the experimental net room at Tokyo University of Agriculture2).

The results demonstrated that plants grow well and can be produced with both good yield and quality when they arc cultivated using nitrogen and phosphoric acid. Additionally, a higher yield and quality can be obtained when the manurial element compost is included. Finally, these experiments have shown that potassium has no influence on the factors studied here.

4.The effects of various kind and amounts of nitrogenous manures on the plant growth, physiology, yield and extracts contents, and ligustilide and butylidene phthalide contents of A. acutiloba were investigated3).

The results showed that the plants can be produced in good yield, displaying both good plant growth and remarkable physiological activity, and can be produced

such that the extracts are maximized, when they are cultivated with ammonium chloride and 2-fold nitroge of standard quantity. Additionally, using gas chromatography (GC), it was established that ligustilide and butylidene phthalide contents in the plants were recovered in a good yield from the fully grown plants in this manner.

5.The effects of various kind and amounts of phosphoric manures on the plant growth, physiology, yield and extracts contents, and ligustilide and butylidene phthalide contents of A. acutiloba were investigated.

The plant growth, the physiological activity, the weight of whole plant, the yield of extract contents, and ligustilide and butylidene phthalide contents were optimal under conditions of mix superphosphate+fused magnesium phosphate and 1 fold phosphoric acid, and dininished gradually with either an increase or decrease in the amount of phosphoric acid. Therefore, mix superphosphate+fused magnesium phosphate and about standard quantity of phosphoric acid seems to be the most suitable condition for "Dang-guei" cultivation.

6.Angelica acutiloba were cultivated under isothermal conditions at 20 ℃ , 25 ℃ and 30 ℃ , and the plants were also cultivated under various temperature ranges at low (20/10 ℃ , day/night). medium (25/15 ℃ , day/night) and high(30/20℃ , day/night) conditions. The results indicated that the plants can be produced in good yield when plants grow well with remarkable physiological activity4, 5).

Ligustilide, a major component in the crude drug, was recovered in a good yield from the fully grown plants, which were cultivated under 20 ℃ or medium (25/15℃ , day/night) temperatures.

7.In order to find the cultural conditions that may improve the growth, yield and quality of A. acutiloba, the effects of the light intensity which the plant was subject to were investigated. The light intensity was controlled with cheese cloth of 40% and 80% shading intensity. The control plants were cultivated without any cheese cloth.

The weight of the individual parts of the plant, the extracts, and the ligustilide contents were found to be heighest under 40% shading conditions. Conversely, 80% shading conditions produced the lowest yield levels, suggesting that the growth was tended to be obstructed under such heavy shading.

Ⅱ .Studies on improving the production and the quality of A. acutiloba Kita-

gawa by controlling the bolting6).

When bolting occurred in A. acutiloba, both plant growth and the physiological activity declined, and once the cambium disappeared and lignification began, the thickening of the root practically came to a halt. At the harvest time, as the plant body was dying, not only was there a marked reduction in production, but also there was a significant decrease in the quality of the crude drug produced.

Until now, the conventional bud hollowing method has been used to control bolting. Unfortunately, this method usually requires much skill and highly advanced techniques, and it takes a lot of working time. The present author has developed the root cutting technique, a new method which, when applied, results in 100% control over bolting development. Additionally, the results of this root cutting treatment indicate that the plants can be produced with good crude drug quality at a good yield. In the future, the mechanization of this new method is a possibility. Such mechanization would reduce the necessary working time, and result in major increase in production and in quality.

Ⅲ .Studies on varietal characteristics and evaluation of different species of Angelica plants by physical chemistry.

1.Varietal characteristics and seasonal variation of plant growth, yield, ether extract and ligustilide contents in several parts of different species of Angelica plants were investigated using thin layer chromatography (TLC) and high-performance liquid chromatography (HPLC)7).

These two methods allowed the classification of different species of Angelica plants into three HPLC, GC pattern groupings:(1)Ofuka, Hokkai, Nichi-touki (Japanese varieties); (2) Dang-guei (Chinese varieties); (3)Kankoku-touki(Korean varieties). There groups corresponded to the three classes: (1)A. acutiloba, (2)A. senensis and (3)A. gigas, based on plant form.

The growth of the aerial part in A . gigas was faster than in the other species, and the ether extract content was also higher. However, since the TLC analysis showed that A. gigas contained no ligustilide, it became evident that the crude drug of A. gigas is completely different from that of A. acuhloba.

Seasonal variation of ligustilide contents contained in the leaf and petiole were measured during the growing period. The ligustilide content in A. acutiloba gradually increased during the period from July to September. The ligustilide

content in the root of A. acutiloba increased to reach its maxima in July. The ligustilide content in the leaf, petiole and root of A. acutiloba var, sugiyamae was highest between May and July, then decreased to its lowest level between September and harvest time (November).

2.Studies on high-performance liquid chromatographic separation and quantitative determination of ligustilide in the Angelica plant using fluorometric detection8, 9).

Gas chromatography has generally been employed in recent quantitative analytical studies of ligustilide and its relative compounds in crude drugs. Ligustilide boils at 168～169 ℃ under 6mm/Hg pressure, therefore the column oven temperature should be set above 190℃ . It can be suggested that this condition is insufficient for the thermally unstable analyte(s) .

Ligustilide has a absorption band at 320 nm and also produces a remarkable bluish fluorescence under excitation at 365 nm. Unfortunately, it decomposes rapidly upon exposure to air and the above absorption band shifts to a shorter wavelength, but remains fluorescent. The present author has applied grating fluorophotometric detector to an HPLC analysis as an advantageous method. The excitation Wavelength was fixed at 365 nm. The total fluorescence produced from a column effluent was first passed through the UV-cut filter and then collected by a zero-nm setting of a fluorescence selection monochrometer (zero-order reflection of the light).

3.A high-performance liquid chromatographic method was applied to the separation of plant sterols in different Angelica Radix.

The results showed that from the n-hexane extracts of Angelica Radix, four kinds of sterols (β -sitosterol, stigmasterol, cholesterol and campesterol) were separated.

4.Thin layer chromatographic and high-performance liquid chromatographic methods were applied to the separation of coumarins in several parts of different Angelica plants.

The TLC and the HPLC analysis results showed that bergapten, umbelliferone, scopoletin, xanthotoxin were present in the fruits, leaves, petioles, roots of A. acutiloba and A. acutiloba var, sugiyamae. However, isopimpinellin is a characteristic component of only the fruits of A.acutiloba and A. acutiloba var, sugiyamae.

Umbelliferone and scopoletin were found to be present in the fruits, leaves, petioles and roots of A. gigas only. Imperatorin is a major and characteristic component in the

fruits of A. gigas. but bergapten is nonexistent in this plant. The coumarins contained in the roots of A. sinensis were found to contain only bergapten and scopoletin.

5.Investigations of the inorganic constituents in different A. Radix were analyzed by using inductively coupled plasma atomic emission spectrometers.

The results indicated that the Mg, Ca, P, K contents were the highest. The main inorganic constituents in different A. Radix were also evident, with Fe, Al, Na, Si contents being somewhat higher than the others. Athough Li, Sr, Mn, Cu, Zn, V, Cr, Ni, Sn, Se, Ba, Sb, Ti, B contents were low, they could nevertheless provide micro inorganic constituent information for their indentification. Be, Pb, Cd, Co, AS, Mo, Ge, Ga, Bi, Sc, Tl contents, however, were non-existent in A. Radix.

Ⅳ .Somatic embryogenesis and plant regeneration from immature and mature seeds in Angelica acutiloba Kitagawa(10, 11).

In vitro reponses to hormones of immature and mature seeds of Angelica acutiloba Kitagawa were studied. Seeds were collected on the 10th, 20th and 30th day after flowering,stripped of the seed costs and cultured on the full and half-strength Murashige and Skoog (MS) or N6 media supplemented with various combinations of auxins (Picloram or 2,4-D) and cytokinins (BA or kinetin).

Immature and mature seeds gave differential responses to these hormones, Only the mature seeds collected on the 30th day after flowering germinated. N6 medium supplemented with 0.1mg/l picloram and 0.5mg/l BA was found to be the best for induction of somatic embryogenesis and subsequent plant regeneration of mature seeds collected on the 30th day after flowering.

Histological studies revealed that the somatic embryos were induced even from the embryos of immature seeds collected on the 20th day after flowering. Complete plants were regenerated from the somatic embryos,and grew in the fields without any phenotypic abnormalities.

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Studies on the Production and the Improvement in Quality of *Angelica Acutiloba* Kitagawa

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Abstract

The study was mainly divided into four divisions to research *Angelica acutiloba* Kitagawa. Firstly, the investigation focused on the relationship of the cultural conditions to the character of plant growth, yield and quality. A series of physiological and ecological studies from seed production, raising of seeding, manuring practice and environment condition etc. to establish optimum cultivation. Secondary, to establish control the bolting method. When bolting occurred in *A. acutiloba*, both plant growth and the physiological activity declined, and once the cambium disappeared and lignification began, the thickening of the root practically came to a halt. At the harvest time, as the plant body was dying, not only was there a marked reduction in production, but also there was a significant decrease in the quality of the crude drug produced. The study author has developed the root cutting technique, a new method which, when applied, results in highly percent control over bolting development. Additionally, the results of this root cutting treatment indicate that the plants can be produced with good crude drug quality at a good yield. Thirdly, to evaluation of quality by physical chemistry. Separated and purified ligustilide, a marker component from *A. acutiloba* seed, and to establish high-performance liquid chromatographic separation and quantitative determination of ligustilide in the Angelica plant using fluoremetric detection. Another, thin layer chromatographic and high-performance liquid chromatographic methods were applied to the separation of sterols (?-sitosterol, stigmasterol, choles-

terol, campesterol) and coumarins (bergapten, umbelliferone, scopoletin, xanthotoxin, isopimpinellin, imperatorin) in several parts of different Angelica plants.

Finally, to develop on the mass propagation of *A. acutiloba* through tissue culture. Culture samples with seeds, leaf, petiole, immature and mature, and cultured on the different media supplemented with various combinations of hormones, the results showed that complete plants were regenerated from the somatic embryos, and grew in the fields without any phenotypic abnormalities.